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PRINCIPAL INVESTIGATOR: Meei-Huey Jeng, Ph.D.

CONTRACTING ORGANIZATION: University of Virginia
Charlottesville, Virginia 22906

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13. ABSTRACT *(Maximum 200*

Women with estrogen receptor positive breast tumors respond to adjuvant hormonal therapy with an enhancement of disease free survival and overall survival. A substantial fraction of tumors are dependent upon estrogen for growth, and antiestrogen has been shown to be effective for the treatment of breast cancer The precise mechanism of estrogen and anti-estrogen action in breast tumors still remain unclear. We postulated that estrogen and anti-estrogen act by activating the function of the endogenous estrogen receptor. Using rat mammary gland as the model, we examined the function of ER in response to estrogen and antiestrogen. Our data suggested that estrogen and anti-estrogen are able to activate and regulate the function of estrogen receptor in a cell context dependent fashion. We conclude that estrogen and anti-estrogen may exert their effects on the growth of target tissues, at least in part, by increasing the functional activity of the endogenous estrogen receptor. Abstracts for the research done in this funding period were submitted to the Annual Endocrine Meeting in June, 1997 and Era of Hope DOD BCRP Meeting in October, 1997 for presentation.

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FOREWORD

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TABLE OF CONTENTS

5. INTRODUCTION

The subject of the current studies is to establish an in vivo target gene transcription activation model in rat mammary gland and uterus for the mechanistic studies of hormones and antihormones in normal and neoplastic cells.

The purpose of these studies is to develop adenoviral reporter as a tool to deliver reporter into rat mammary gland and to examine whether hormones or antihormones can activate target gene transcription activation in an in vivo setting where cell-cell interaction occurs. This novel approach will answer questions where traditional method cannot. Traditional method using tissue culture cells for the study of hormone actions cannot answer the position effect occurs on estrogen target organs where multiple cell types exist. In addition, if one can deliver reporter into animal tissues, one can examine the multiple effects of hormones and antihormones on various estrogen target organs such as mammary gland and uterus. The success of the development of the adenovirus approach to deliver reporter into animal organs will allow us to study the agonist and antagonist effects of antiestrogens in different organs.

The scope of the research is focused on the testing and optimizing the condition for delivering the adenovirus reporter into rat mammary gland. We will use rat mammary gland as the model system to establish the in vivo target gene transcription activation in order to examine the action of estrogen and antiestrogen at the molecular level.

The background of previous work: Women with estrogen receptor (ER) positive breast cancers respond to adjuvant hormonal therapy with an enhancement of disease free survival and overall survival. A substantial fraction of tumors are dependent upon estrogen for growth. Antiestrogen has been shown to be effective for the treatment of breast cancer. The precise mechanism of estrogen and anti-estrogen action in breast tumors still remain unclear. Tissue culture studies have suggested that the binding of estrogen to ER and the subsequent activation of ER target genes are involved in the estrogen and anti-estrogen regulated cell proliferation. However, the function of ER in mediating the estrogen and antiestrogen actions in breast tumors or in estrogen target organ such as the mammary gland has never been addressed due to the technical difficulty. To better understand the mechanism of estrogen and anti-estrogen action in a physiological environment where cell-cell interaction, stromal-epithelial interaction, and the contribution of cell context exist, we attempt to develop a novel tool for the delivery of reporter in vivo in estrogen target tissues. We postulate that the activation of ER target gene is cell context dependent in mammary gland and anti-estrogen works through inhibition of the transcriptional activation of ER target genes in vivo. Rat mammary gland was used as the model system to study the estrogen and anti-estrogen actions on regulation of ER target gene because both estrogen and anti-estrogen have been shown to regulate mammary gland development and carcinogenesis process.

The wide use of antiestrogen tamoxifen as adjuvant therapy in human breast cancer patients has prompted us to examine the potential effect of tamoxifen on various estrogen target organs. It has been documented that tamoxifen has antagonist effect on breast tissue. However, it is estrogenic on bone to prevent osteoporosis and estrogenic on virgina to increase the incidence of endometrial cancer. To understand why a single compound will have different effect on various target organs, we need a model system to measure the mechanistic action of antiestrogen in these organs. Previously, we have demonstrated that adenovirus can infected rat mammary gland with high infectivity and the inducible adenoviral reporter can be activated in MCF-7 cells grown in culture by estrogen treatment. This allows us to use adenovius as delivering tool to infect mammary gland with genes we are interested. The current studies address the efforts to test adenoviral reporter in rat mammary gland. We have successfully establish the in vivo infection of rat mammary gland with adenoviral reporter and demonstrated that estrogen and antiestrogen can regulate the target gene tranascription activation in vivo. We hope that a precise measurement of actions of steroid hormones and antihormones on target tissues will provide a rational for the development of new preventive approaches for controlling the increased incidence of breast cancer.

6. BODY

a. EXPERIMENTAL METHODS

Various adenoviral reporters were constructed and screened for their ability to transactivate the target gene function. These viruses were injected into rat mammary gland and tested for their transactivation function by estrogen and antiestrogen.

To infuse the reporter into rat mammary gland, a vital tracking dye was used to monitor the success of infusion in conjunction with adenovirus. Rats were first anesthetized and the main duct was cannulated with a blunt-ended needle and infused with indigo carmine tracking dye. The dye difused into mammary gland in less a second.

To assess the transactivation function of ER using adenoviral infection approach, Wistar Furth 40 day old female rats were ovariectomized in order to eliminate endogenous estrogen and progesterone levels. Ten days after ovariectomy, #3 thoracic and #4 abdominal mammary glands were cannulated with a blunt-ended needle and infused with inducible reporter virus ad-ERE-tk-βgal. Estrogen was given at 0.1, 1, 10, and 100 μg per rat and anti-estrogen ICI 182,780 was administered at 100 μg per rat. Ad-CMV-βgal was also infused to assess the infection efficiency. Two days after injection of the reporter virus, the fat pad will be excised and stained with X-gal. Serum was collected to determine the circulating estradiol concentration and uterine wet weight was also measured as a positive control.

b. ASSUMPTIONS

Combining the intraductal injection and adenoviral reporter strategies, we assume that the adenoviral reporter will infect rat mammary epithelial cells with high infectivity. This novel strategy will allow us to assess the transactivating function of ER in a physiological condition in an estrogen target organ. Unlike the tranditional method using tissue culture transfection to assess the transactivating function of ER, our model system allows one to examine the molecular mechanism of hormonal action in a tissue organ.

c. PROCEDURES

The approach taken was to deliver a reporter gene, β-galactosidase, under the control of estrogen responsive element (ERE) to transfect mammary epithelium in vivo via the main duct using adenovirus as the carrier. Female Wistar-Furth rats were anesthetized and ovariectomized to reduce the endogenous estrogen level. Mammary main ducts were cannulated with blunt-ended needle and infused with adenoviral vector expressing β-galactosidase under the control of ERE and thymidine kinase promoter (Ad-ERE-tk-βgal) in conjunction with the tracking dye indigo carmine blue. Estrogen benzoate or anti-estrogen ICI 182,780 were injection S.C. at various concentrations. Two days later, mammary fat pads was dissected, fixed, and stained with X-gal to visualize the reporter activation *in vivo*. To avoid the strain-dependent reporter activation, two other strains of rats, Sprague-Dawley and Holtzman, were also used in this study. Serum was also collected to determine the concentration of circulating estrogen using radioimmuno assay, and uterine horn was dissected and the wet weight measured.

d. RESULTS AND DISCUSSION

Our results demonstrated that estrogen was able to activate the ER target gene transcription*in vivo* in rat mammary gland and this activation occurred in a dose dependent fashion (1 & 2) and coincided with the circulating estrogen level (Table 1). The Ad-ERE-tk-βgal could be transcriptionally activated, but not Ad-ERE-tk-βgal, by estrogen treatment in rat mammary gland model. Interestingly, the reporter activation occurred predominantly in lobuloalveolar epithelial cells, but not in primary ductal epithelial cells. Similar results were also obtained in other strains of female Sprague-Dawley and Holtzman rats. More importantly, treatment of ICI 182,780 was able to block the estrogen-induced target gene transcriptional activation *in vivo* (1 & 2). The blockade of estrogen-induced reporter activation by ICI 182,780 could be reversed by an increased concentration of estrogen. In summary, we have demonstrated the ability of estrogen and anti-estrogen to regulate the target gene activation at the transcriptional level *in vivo.*

Tasks 1, 3, 4, and 5 have been completed. task 2 still needs time to construct other adenoviruses, Ad-PRE-tk-βgal and Ad-CMV-CAT due to the delay of establishing a new research program at the University of Virginia. Task 6 and 7 has been started to deliver adenoviral reporter into uterus. Task 8, 9, and 10 are expected to be completed during the second funding year. A grant proposal based on the data generated from this research proposal is also on the plan for a RO-1 submission in the near future.

Table 1. Mammary gland ER transactivation, serum estradiol concentration, and uterine wet weight in rats treated with various doses of 17-β estradiol benzoate (EB) and mammary glands infused with Ad-ERE-tk-βgal

Treatments	ER transactivation in mammary gland	serum estradiol conc., pg/ml	uterine wet weight, mg
Control	+	20.4 ± 0.4	36.7 ± 2.9
EB, 1 μg/kg	++	21.6 ± 1.6	79.3 ± 10.5
EB, 10 μg/kg	++++	23.3 ± 0.1	149.0 ± 23.1
EB, 100 μg/kg	++++	53.0 ± 13.7	140.9 ± 18.1
EB, 1000 μg/kg	++++	706.1 ± 0	123.9 ± 8.6

e. RECOMMENDATIONS IN RELATION TO THE STATEMENT OF WORK

task 2: for the construction of reporter adenoviruses need to be extended due to large amount of work involved which was underestimated initially. PI has been the only manpower involved in this project and the establishment of new research program at the University of Virginia for this PI's first research project also delayed the progress due to the time involved to setup the laboratory. However, the overall progress has been moving along well and is heading toward the tasks planned for year two of this research project period.

7. CONCLUSIONS

We conclude that estrogen and anti-estrogen may exert its effects of the growth and proliferation in target tissues, at least in part, by increasing the functional activity of the endogenous ER. Similar approach of adenoviral delivery of reporter into estrogen organs such as the uterus and bone can be achieved for the study of the mechanism of estrogen, anti-estrogen, and the modifiers of ER action. We also predict that the measurement of the function of ER is more important and sensitive than the measurement of the actual ER protein level as the prognosis factor for breast cancer treatment. We hope that this study will provide a basis for the future possible inclusion of the ER functional measurement in breast cancer prognosis.

8. REFERENCES

1. Jeng, M-H, Santen, RJ, Conneely, OM, et al. Blockade of the estrogen-induced transcriptional activation of estrogen receptor target gene by anti-estrogen in rat mammary gland. 79th Annual Meeting of the Endocrine Society, June 11 - 14, 1997.

2. Jeng, M-H, Conneely, OM, O'Malley, BW, Santen, RJ, Kao, C. Estrogen receptor function in vivo: transcriptional activation of the estrogen receptor target gene by estrogen and antiestrogen in rat mammary gland. Era of Hope at the Department of Defense Breast Cancer Research Program Meeting, October 31 - November 4, 1997.

9. APPENDICES

None